REMARKS

Claims 49-61 and 63-66 are pending and are rejected under 35 U.S.C. § 103. New claims 67-72 are added.

Support for the Amendments

Support for new claims 67-72 is found in the specification and claims as originally filed. In particular, support for new claims 67, 68, and 70, which recite GM-CSF, is found in Applicants' specification at page 4, line 32; support for new claims 68 and 69 is found at claim 1 as originally filed; support for new claim 69, which recites "an effective amount of a cytokine that mobilizes endothelial progenitor cells" is found, for example, at page 27, lines 27-27;" support for new claim 71, which recites "stem cell factor (SCF)" is found at page 5, line 1; and support for new claim 72, is found at page 20, lines 15-22.

Priority Claim

The Office objects to Applicants' priority claim, and asserts that the pending claims are entitled only to the effective filing date of the current application. In support of this assertion, the Office alleges that Applicants' U.S. provisional application, which was filed on March 9, 1998, (U.S.S.N. 60/077,262, hereinafter "the '262 application") discloses only the administration of granulocyte macrophage colony stimulating factor in combination with an angiogenic protein, and that it lacks support for other factors. Applicants respectfully disagree with the present rejection.

While the Office acknowledges that the '262 application specifically describes the use of GM-CSF to enhance endothelial progenitor cell mobilization, Applicants' invention is not so limited. Applicants broadly disclose that cytokine-induced endothelial progenitor cell mobilization enhanced neovascularization of ischemic tissues. For example, at page 13, lines 13-15, where Applicants state that "cytokine-induced EPC mobilization can enhance neovascularization in both severe tissue ischemia, as well as do novo (sic) vascularization of previously avascular tissues." Clearly, the scope of Applicants' invention is not limited to GM-CSF, but extends to virtually any cytokine that induces EPC mobilization. Methods for identifying such factors are clearly described in the '262 application, as evidenced at pages 12 and 13, under the heading "Modulation of EPC kinetics by cytokines," where Applicants broadly

disclose that cytokine administration mobilizes endothelial progenitor cells (EPCs) thereby augmenting therapeutic neovascularization as well as describing methods for identifying such factors. These methods include the mouse corneal pocket assay and the rabbit hindlimb ischemia assay. Applicants further disclose that "an angiogenic factor can be combined with other genes or their encoded gene products to enhance the activity of targeted cells, while simultaneously inducing angiogenesis." A list of exemplary angiogenic factors that are useful in the methods of the invention is provided at page 8, lines 6-16. Given that Applicants' priority application broadly discloses that cytokines that mobilize endothelial progenitor cells (EPCs) are useful for the treatment of myocardial ischemia, the Office's objection to the priority claim should be withdrawn.

In order to expedite prosecution and facilitate allowance, Applicants note that new claim 68 is directed to methods of treating myocardial ischemia by administering to the mammal an effective amount of granulocyte macrophage colony stimulating factor (GM-CSF) or an effective fragment thereof; and administering an effective amount of a nucleic acid encoding at least one angiogenic protein or an effective fragment thereof into the myocardial tissue; thereby treating the ischemic myocardial tissue of the mammal. Support for the administration of GM-CSF is found in the '262 application at page 4, lines 27 and 28, where Applicants state "These methods involve the use of GM-CSF to mobilize endothelial cell (EC) progenitors." Given that the '262 application clearly discloses methods of treating myocardial ischemia by injecting a nucleic acid encoding an angiogenic protein and administering to the mammal an effective amount of granulocyte macrophage colony stimulating factor, the objection to the priority claim does not apply to claim 67.

In addition, Applicants have added new claim 68 to clarify that the invention is broadly directed to methods of treating myocardial ischemia by administering to a mammal an effective amount of a cytokine that mobilizes endothelial progenitor cells; and subsequently administering an effective amount of a nucleic acid encoding at least one angiogenic protein or an effective fragment thereof into the myocardial tissue; thereby treating the ischemic myocardial tissue of the mammal. The '262 application plainly provides support for new claim 68 at page 13, lines 13 and 14, where Applicants state "cytokine-induced EPC mobilization can enhance neovascularization" and at page 7, lines 18-21, where Applicants state:

If it is desirable to further enhance angiogenesis, **angiogenic proteins, e.g., endothelial cell mitogens, may also be administered to the patient in conjunction with, or subsequent to, the administration of the GM-CSF**. The angiogenic protein can be administered directly, e.g., intra-arterially, intramuscularly, or intravenously, or nucleic acid encoding the mitogen may be used.

Accordingly, the Office's objection to the priority claim clearly does not apply to new claims 68 and 69.

Rejections under 35 U.S.C. § 103(a)

The Office rejects claims 49-61 and 63-66, which are directed to methods of treating an ischemic myocardial tissue by injecting GM-CSF or stem cell factor (SCF) and a nucleic acid encoding an angiogenic protein, under 35 U.S.C. § 103(a) as obvious over WO 97/14307 by Isner (hereinafter "Isner"), in view of U.S. Patent No. 5,880,090 by Hammond et al., (hereinafter "Hammond"); these claims are further rejected over Isner in view of Bussolino et al., (J. Clin. Invest. 87:986-995, 1991). Applicants respectfully disagree and for the reasons detailed below request that the obviousness rejection of the pending claims be withdrawn.

The test of obviousness requires that one compare the claimed "subject matter as a whole" with the prior art "to which said subject matter pertains" 35 U.S.C. § 103(a). To establish a *prima facie* case of obviousness, three criteria must be met. First, a suggestion or motivation to modify the reference or combine reference teachings must be present in the references or in the general knowledge present in the art. Second, there must be a reasonable expectation of success. Finally, the prior art reference must teach or suggest all the claim limitations. M.P.E.P. 2143. The burden is on the Office to show that the references expressly or impliedly suggest all of the claim limitations. M.P.E.P. 2142. "There are three possible sources for a motivation to combine references: the nature of the problem to be solved, the teachings of the prior art, and the knowledge of persons skilled in the art." *In re Rouffet*, 149 F.3d 1350, 1357. In the absence of some teaching or suggestion to combine, no *prima facie* case of obviousness can be established, and the rejection is improper and must be withdrawn. *In re Fine*, 837 F.2d 1071, 1074.

In the present case, the references cited by the Office fail to provide the requisite motivation to combine; fail to provide a reasonable expectation of success; and fail to teach or suggest all of the claim limitations. Each of the references cited by the Office in support of the obviousness rejection is considered below.

Isner

Isner describes methods for treating ischemic tissue. The Office states:

> ***Isner does not specifically teach the administration of an effective amount of a stem cell factor, a colony stimulating factor or an effective fragment thereof*** into the mammal with an effective amount of a solution comprising a nucleic acid encoding at least one angiogenic protein or an effective fragment thereof. (Office action mailed January 25, 2006; page 4, last paragraph)

Hammond

Hammond describes methods for coating a ***synthetic vascular graft*** with endothelial cells. Regarding Hammond, the Office asserts that i) the methods of Hammond are indistinguishable from those described by Applicants; ii) Hammond teaches methods for enhancing blood vessel formation in a patient; and iii) one of skill in the art would be motivated to combine the methods taught by Hammond with the methods described by Isner. Applicants respectfully disagree with these assertions.

I. Methods for enhancing endothelialization of a synthetic graft are readily distinguishable from methods of treating an ischemic tissue

The methods described by Hammond are uniformly directed to methods for coating ***synthetic grafts*** with endothelial cells; such methods are readily distinguishable from Applicants' claimed methods, which are directed to methods for treating ischemic myocardial ***tissue of a mammal.*** The synthetic grafts described by Hammond comprise polyethylene terephthalate and polytetrafluoroethylene. The usefulness of such grafts is limited by their tendency to induce clot formation (column 1, lines 15-26). To overcome such limitations, Hammond describes methods for increasing the number of endothelial cells that ***attach to and coat the surface*** of synthetic grafts. Hammond states, "circulating cells that give rise to ***endothelial coatings of vascular prostheses*** may arise from the bone marrow. (column 1, line 60, to column 2, line 5; emphasis added; citations deleted.) Methods for increasing the number

of endothelial cells that attach to a graft to form an endothelial coating are distinctly different from Applicants' claimed methods, which provide for the treatment of myocardial ischemia in a ***patient***. The formation of ***endothelial coatings on synthetic grafts*** described by Hammond can in no way make obvious Applicants' claimed methods.

II. Hammond fails to teach methods for enhancing endothelialization in a tissue of a patient.

The Office asserts that Hammond teaches methods for enhancing endothelialization of tissues in a patient. Specifically, the Office states that Hammond showed that "mobilization of endothelial cell progenitors would further enhancing blood vessel formation or angiogenesis in an ischemic tissue." (Office action mailed January 25, 2006, page 8, lines 11-14.) Applicants respectfully disagree. In fact, Hammond fails to describe any method of enhancing endothelialization in a ***tissue or organ*** of a patient, as Applicants do. As detailed above, the methods described by Hammond merely provide for ***endothelial coatings of synthetic materials*** to reduce thrombus formation. (column 2, lines 53-67).

Not only are such synthetic grafts plainly distinct from the tissues of a mammal, but the formation of endothelial coatings is also distinct from the formation of blood vessels within a tissue. Hammond teaches that endothelialization promoting agents (e.g., GM-CSF, G-CSF) enhance "**adherence of circulating endothelial cells to graft surfaces**, or may stimulate the multiplication of blood-borne endothelial precursors that have become **adhered**." (column 2, lines 64-67.) Hammond teaches that this process relies on "'fallout endothelialization.' More specifically, it has been proposed that the circulating cells that give rise to endothelial coatings of vascular prostheses . . . " Methods for coating a synthetic prostheses with a layer of endothelial cells are readily distinguishable from the multifaceted biological process that regulates blood vessel formation within a tissue or organ.

Applicants' specification teaches that the formation of blood vessels in a tissue involves the complex regulation of a variety of endothelial cell functions and activities, including cell migration, proliferation, the formation of endothelial cell sprouts, vascular loop formation, the development of capillary tubes and the subsequent formation of tight junctions and the deposition of new basement membranes (page 2, lines 10-15). Such vascular networks fulfill a critical biological function within the tissue of the subject by providing oxygen and nutrients and

removing wastes (page 1, lines 27-30). Hammond's process of coating a synthetic graft with endothelial cells is plainly different from the process of blood vessel formation.

To expedite prosecution and clarify the distinguishing features of the present invention, Applicants note that new claim 68 recites that the method increases the neovascularization of the tissue. Accordingly, the obviousness rejection over Isner in view of Hammond should not apply to claim 68.

III. Hammond teaches away from combining the methods described by Isner with methods for promoting endothelialization.

The endothelialization results obtained by Hammond fail to provide the requisite motivation to combine or the expectation of success to modify the methods of Isner. A thorough reading of Hammond suggests that methods for promoting endothelialization may have undesired side effects that would dissuade the skilled artisan from utilizing the methods described by Hammond. In particular, Applicants invite the Office's attention to Example 1, where Hammond describes grafts having endothelial coatings. Regarding such grafts, Hammond states,

> [T]he BMB grafts implanted for four weeks or longer appeared stiff. Histological studies revealed many **osteocytes with microcalcification in the outer graft wall** of these grafts, but not in the inner wall or intima, even at three months. **In the BMB grafts implanted longer than four weeks, osteoblasts, osteocytes, and microcalcifications were found. These undesirable side effects could affect the long-term utility of such grafts . . .** (column 7, lines 55-63)

Hammond's disclosure of adverse results associated with the endothelialization of grafts teaches away from the use of such methods. In view of this teaching away, one skilled in the art would lack the requisite motivation to introduce changes to the methods of Isner, and would further lack the expectation of success required to introduce such changes.

In sum, Hammond fails to teach or suggest any method for treating myocardial ischemia in a tissue of a subject, much less Applicants' claimed methods, which recite identifying a patient in need of such treatment, injecting a nucleic acid molecule encoding an angiogenic factor, and administering SCF or GM-CSF. Moreover, one skilled in the art would lack the

requisite expectation of success to combine the methods of increasing synthetic graft endothelialization described by Hammond with any other method described in the references cited by the Office.

Bussolino

Bussolino describes the use of G-CSF and GM-CSF to induce proliferation and migration in endothelial cells. The Office asserts that it would be obvious to modify the methods of Isner by utilizing recombinant G-CSF as described by Bussolino. In support of the rejection, the Office states:

> An ordinary skilled artisan would have been motivated to carry out the above modification because Bussolino et al. already demonstrated that recombinant G-CSF has angiogenic activity *in vivo*, and that it also exhibits synergistic effects with at least another endothelial cell mitogen bFGF in inducing in vivo angiogenesis.

Applicants respectfully disagree.

I. Bussolino shows that G-CSF has only a weak angiogenic effect in vivo, and fails to conclude that G-CSF and bFGF act cooperatively

Contrary to the Office's assertion, Bussolino shows that when G-CSF is administered alone it exhibits only **weak angiogenic activity** *in vivo*. At page 994, right column, lines 2 and 3, Bussolino states, "G-CSF had ***relatively weak***, but definite, angiogenic activity in the rabbit cornea." (emphasis added.) The weak angiogenic activity described by Bussolino would be insufficient to motivate the skilled artisan to adapt the methods taught by Isner by including G-CSF, particularly where Bussolino states that G-CSF was less active than bFGF (page 986, Abstract). Moreover, Bussolino fails to conclude that G-CSF and bFGF exhibit a synergistic effect. Rather, Bussolino states that the observed effects are ***merely suggestive*** of a cooperative effect. At page 994, lines 12-15, Bussolino states, "By combining nonangiogenic doses of bFGF with G-CSF, we observed responses whose intensity is ***suggestive*** of a cooperative interaction of the two cytokine in inducing angiogenesis (emphasis added). Thus, Bussolino fails to conclusively determine the nature of the interaction between bFGF and G-CSF.

II. Bussolino emphasizes the preliminary nature of the G-CSF and bFGF results

In addition, Bussolino fails to teach or suggest that G-CSF should be used to enhance angiogenesis in other tissues. In fact, Bussolino indicates that further studies are required to characterize the effects of G-CSF on angiogenesis. Rather than concluding that G-CSF should be used to enhance angiogenesis in any tissue, Bussolino repeatedly emphasizes the preliminary nature of the G-CSF and bFGF results. Bussolino states, "we wanted to obtain ***initial indications*** as to the capacity of this cytokine to act in concert with bFGF." Bussolino also states that "This ***initial observation*** needs to be ***extended***." Clearly, Bussolino indicates that his results are not the endpoint of exhaustive studies, but are merely preliminary indications, which provide a jumping off point for further study.

III. Bussolino teaches that it is difficult to predict effects on *in vivo* angiogenesis

Nor does Bussolino indicate that these studies are broadly applicable to methods for enhancing angiogenesis in a variety of tissues *in vivo*. In fact, Bussolino stresses that it is difficult to predict effects on *in vivo* angiogenesis given the complexity of the biological processes involved. Bussolino states:

> *In vivo* angiogenesis occurs as the end point of ***complex interactions*** between many events involving the remodeling of the extracellular matrix and the release of several "factors". ***This apparent paradox of a combination of cytokines acting directly on endothelial cells, showing a cooperative effect in vivo, but not in vitro,*** adds to the list of factors or conditions for which ***in vitro modulation of proliferation and migration is not necessarily predictive of in vivo effects on angiogenesis.*** Possible explanations for this partial ***discrepancy in the capacity of G-CSF to act in concert with bFGF in vitro and in vivo*** could involve a different biology of microvascular endothelium versus HUVEC or ***effects of G-CSF on passing neutrophils.***

Bussolino indicates that his observations concerning angiogenesis are influenced by complex and unpredictable interactions that are liable to be ***influenced even by the effects of passing neutrophils***. Given the paradoxes and uncertainties that exist in the results of Bussolino, Bussolino fails to provide the motivation or expectation of success required to adapt the methods of Isner.

The standard in determining obviousness is not whether certain experiments ***could be tried***, but whether the prior art suggested that the modifications ***should be made***, and further

suggested that the modified methods ***would function successfully***. *In re Dow Chemical Co.*, 837 F.2d 469 (Fed. Cir.,1988). In this case, the court held:

> The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that ***this process should be carried out*** and would have ***a reasonable likelihood of success***, viewed in the light of the prior art. Both the suggestion and the expectation of success must be founded in the prior art not in the applicant's disclosure. *Id* at 473. (citations omitted; emphasis added.)

None of the references cited by the Office teaches or suggests each of the elements recited in Applicants' claimed invention. None teaches that one should administer GM-CSF or SCF in combination with an angiogenic factor for the treatment of an ischemic myocardial tissue. It is not sufficient that one ***could*** have made the combination, the cited references must suggest the desirability of making the claimed combination and must further indicate that the combination if made would have succeeded.

In sum, Applicants were the first to appreciate that myocardial ischemia could be treated by injecting a myocardial tissue with a nucleic acid encoding an angiogenic factor and administering SCF and GM-CSF. None of the references cited by the Office, alone or in any combination, teaches or suggests all of the claimed limitations of Applicants' claimed invention. The Office has failed to establish a *prima facie* case of obviousness, and the rejection of the claims under U.S.C. § 103(a) should be withdrawn.

Double Patenting Rejection

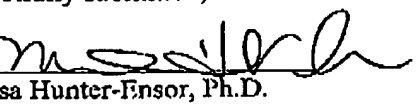
Claims 49-61 and 63-66 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 49, 52-56, 58-65, and 68 of co-pending U.S. Application No. 10/696,391. Applicants submit that upon consideration and entry of the instant Amendment and Response, the provisional double patenting rejection will be the only rejection remaining in the instant application. Therefore, pursuant to M.P.E.P. §822.01, Applicants respectfully request that the provisional obviousness-type double patent application be withdrawn so that the instant application may proceed to allowance.

CONCLUSION

In view of the above remarks, Applicants believe the pending application is in condition for allowance. Accordingly, the Office is respectfully requested to pass this application to issue. Should any of the claims not be found to be allowable, Applicants respectfully request the Office to telephone Applicants' undersigned representative at the number below so that a telephonic interview may be scheduled. Applicants thank the Office in advance for this courtesy.

Dated: June 26, 2006

Respectfully submitted,

By 

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